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GASTROENTEROLOGY 68: 1485–1491, 1975

Vol. 68, No. 6
Printed in U.S.A.

ANALYSIS OF THE MOTOR EFFECTS OF 13-NORLEUCINE MOTILIN ON THE RABBIT, GUINEA PIG, RAT, AND HUMAN ALIMENTARY TRACT IN VITRO

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Motor effects of 13-norleucine motilin (13-Nle-M), a synthetic analog of motilin and biologically equivalent to the natural polypeptide, on the rabbit, guinea pig, rat, and human alimentary tract were investigated in vitro. Whereas guinea pig and rat preparations proved refractory to 13-Nle-M action, muscle strips of the stomach and upper small intestine from rabbit and man were highly sensitive to 13-Nle-M, contractile responses being elicited with concentrations of less than 2×10^{-9} M. Although circular muscle from rabbit colon responded to 13-Nle-M, *Taenia coli* preparations were unaffected by the polypeptide; in man, the reverse was observed. Gallbladder, uterine, and vascular smooth muscle were unresponsive to 13-Nle-M. Pharmacological analysis revealed that the effects of 13-Nle-M on the gastrointestinal muscle are not mediated via nervous pathways: ganglion blockade by hexamethonium, blockade of axonal conduction by tetrodotoxin, or anticholinergic action by atropine failed to affect 13-Nle-M actions. It was therefore concluded that 13-Nle-M caused contractions by stimulating receptors on or in the muscle cell. By use of the antihistaminic pheniramine, histamine receptors could be differentiated from the site of 13-Nle-M action. As the contractile response to 13-Nle-M was abolished by the Ca^{++} antagonistic compound verapamil, a role for 13-Nle-M in the transport of Ca^{++} to the cytosol of intestinal smooth muscle might be considered.

Motilin, a linear polypeptide containing 22 amino acid residues, was isolated from the mucosa of the upper meter of small intestine of the hog.[2, 3] As motilin increases motor activity in both antral and fundic gland area pouches of the stomach of dogs,[2] it was postulated to be involved in the hormonal regulation of gastrointestinal motility.[4] Recently, 13-norleucine motilin (13-Nle-M) was synthesized[5] and shown, at least in dogs, to be biologically equivalent to the natural polypeptide (J. C. Brown, *unpublished observations*).

The present study deals with 13-Nle-M actions on the contractility of the alimentary tract of rabbit, guinea pig, rat, and man in vitro. Evidence is provided that motilin effects are restricted to specific regions of the gastrointestinal tract, and that gallbladder, uterus, and vascular smooth muscle are not affected by the polypeptide. Moreover, pharmacological analysis reveals that the mode of action of 13-Nle-M on gastrointestinal smooth muscle is a direct one.

Received July 22, 1974. Accepted December 12, 1974.

Part of this work has been published as a short communication.[1]

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Materials and Methods

Male rabbits weighing about 2 kg were killed by a blow on the neck. Guinea pigs (body weight 200 to 300 g) and Wistar rats (body weight 180 to 200 g) of either sex were etherized, and the gastrointestinal tissue was resected and used instantly. Human material was taken from macroscopically normal parts of gut removed at operations for various gastrointestinal diseases. Uterine muscle was prepared from female rabbits and rats showing estrus phase on vaginal smear. After removal of mucosa and submucosa, muscle strips (measuring approximately 4 by 15 mm) from body and antrum of the rabbit, guinea pig, rat, and human stomach were cut parallel to the longitudinal or circular layers. From small intestine (species: rabbit, guinea pig, rat) and large intestine (species: rabbit, guinea pig; *Taenia coli* discarded), segments of 15-mm length were dissected parallel to the longitudinal axis. In the rat, large intestinal strips were cut parallel to the longitudinal and the circular portions of the muscularis, respectively. In man, circular muscles were dissected from small and large intestine. *Taenia coli* was prepared from the transverse and descending part of the rabbit, guinea pig, and human colon. The gallbladder was isolated from rabbits, and muscle strips 8-mm long and 2-mm wide were obtained; human strips measured approximately 15 by 4 mm. The descending thoracic aorta was removed from rabbit and rat and cut along a close spiral essentially as described by Furchgott et al.[6] Blood pressure was measured in rabbits anesthetized with urethane (1 g per kg of body weight) by means of a Statham pressure transducer via a plastic cannula introduced into the carotid artery.

Muscle strips were mounted along their long axis in an organ bath containing 10 ml of modified Tyrode's solution at 37°C and continuously bubbled with 5% carbon dioxide in oxygen. The solution had the following composition (g per liter): NaCl, 8.0; KCl, 0.26; $MgCl_2 \cdot 6H_2O$, 0.211; $NaHCO_3$, 1.0; NaH_2PO_4, 0.055; $CaCl_2 \cdot 2H_2O$ 0.255; glucose, 1.0. Isotonic contractions of strips were recorded by means of a writing lever, usually magnified 7 times and preloaded with 1 g, on a Kymograph (model 2050, B. Braun Melsungen, West Germany). When gallbladder strips from rabbit were used, the writing magnification was 20 times under 0.5 g of tension. Isometric recordings were obtained only with uterine smooth muscle, using a force displacement transducer (model HSE-K 30, Hugo Sachs Elektronik K.G., Hugstetten, West Germany) connected to a Multi-Pen Recorder (model B34, RikaDenki Elektronik GmbH, Freiburg, West Germany); the strips were adjusted to an initial tension of 0.5 g. All preparations were allowed a period of 30 min for equilibration until a steady base line and full spontaneous activity were established. Prior to addition of test substances, each strip was subjected to repeated stimulation with acetylcholine and oxytocin, respectively, until a reproducible response was obtained.

The following drugs were used. The 13-norleucine analog of motilin (13-Nle-M) was synthesized by the conventional method described in detail by Wünsch et al.[5] When tested for stimulatory activity in conscious dogs provided with antral and fundic gland area pouches, 13-Nle-M (corresponding to fraction Mo-C_1 of the purification process) was found to be equipotent to the natural polypeptide according to indices of motor activity[7] obtained (J. C. Brown, *unpublished observations*). Other chemicals used included acetylcholine chloride (Hoffmann-La Roche AG, Grenzach, West Germany), histamine hydrochloride (Schuchardt GmbH, Munich, West Germany), pheniramine (Albert-Roussel Pharma GmbH, Wiesbaden, West Germany), verapamil (2,9-bis-[3,4-dimethoxyphenyl]-2-isopropyl-6-methyl-6-azanonanenitrile; Knoll AG, Ludwigshafen, West Germany), which is thought to inhibit the influx of calcium ions into the intracellular space thus preventing the activation of myofibril adenosine triphosphatase,[8] tetrodotoxin (Sankyo Company Ltd., Tokyo, Japan), atropine sulfate, nicotine base, and hexamethonium iodide (E. Merck AG, Darmstadt, West Germany). Solutions were prepared immediately prior to use. Concentrations of the drugs are expressed in terms of their salts; nicotine was used as the base.

Results

Profile of Contraction and Concentration-Response Relationship for 13-Nle-M

Rabbit isolated duodenum proved particularly sensitive to 13-Nle-M, resulting in tonic contraction of smooth muscle without increasing amplitude or frequency of spontaneous actions. The contraction by 13-Nle-M was rapid in onset, and relaxation of the preparation occurred only slowly after washing out (fig. 1). Without washout, contractions were maintained for a long period: contraction amplitude decreased by only 50% after 30 min. Figure 2 shows a mean concentration-response relationship

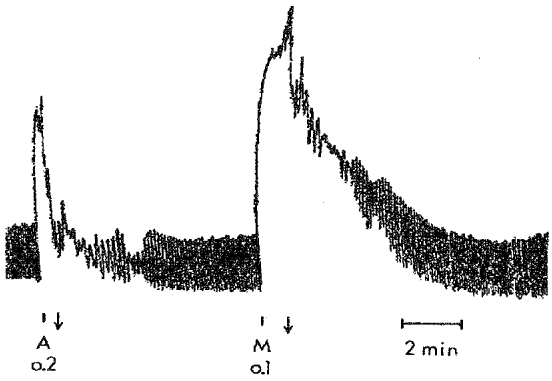

FIG. 1. Responses of rabbit isolated duodenum to acetylcholine (*A*, 0.2 × 10^{-6} g per ml) and to 13-Nle-motilin (*M*, 0.1 × 10^{-6} g per ml). *Vertical bar*, addition of drug to the bath fluid. *Inverted arrow*, washout of drug.

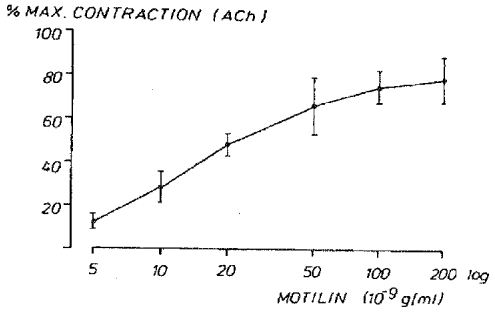

FIG. 2. Log concentration-response curve for 13-Nle-motilin on rabbit isolated duodenum. Contractile responses in percentage of maximal contraction attained with 10 × 10^{-6} g of acetylcholine (*ACh*) per ml. Each point is the mean of four experiments ± SD.

for 13-Nle-M on rabbit isolated duodenum. The threshold concentration of 13-Nle-M, which still produced an increase in the amplitude of spontaneous contractions, was as low as 5 × 10^{-9} g per ml, corresponding to less than 2 × 10^{-12} in molar concentration.

Action of 13-Nle-M along the Rabbit Alimentary Tract

Stomach. Strips of body muscle (n = 10) were insensitive to 0.5 × 10^{-6} to 2 × 10^{-6} g of 13-Nle-M per ml. As expected, the tissue was sensitive to acetylcholine. Antral circular muscle (n = 15) responded to 13-Nle-M (fig. 3).

Small intestine. Duodenal strips (n = 26; fig. 2) and jejunal strips (n = 10) contracted sensitively to 13-Nle-M, the lowest effective concentration tested being 5 × 10^{-9} g per ml.

In 3 of 12 experiments, terminal ileum preparations responded to 13-Nle-M when concentrations as high as 2 × 10^{-6} g per ml were used (fig. 4). No ileal response occurred with lower doses of the polypeptide.

Large intestine. Whereas *Taenia coli* preparations (n = 6) were insensitive even to 10 × 10^{-6} g of 13-Nle-M per ml, circular muscle of descending colon (n = 8) responded to 13-Nle-M concentrations as low as 20 × 10^{-9} g per ml.

Action of 13-Nle-M on Rabbit Smooth Muscle of Other than Gastrointestinal Origin

Gallbladder, uterus, and vascular smooth muscle. Strips of gallbladder (n = 4) and uterine smooth muscle (n = 2) gave no contractile response even to 10^{-6} and 10 × 10^{-6} g of 13-Nle-M per ml, respectively. Aortic strips (n = 4) did not contract to 10^{-6} g of 13-Nle-M per ml. Catheterization of the carotid artery revealed no effect of 13-Nle-M (10 × 10^{-6} g as an intravenous bolus) on blood pressure.

Lack of Effect of 13-Nle-M along the Guinea Pig and Rat Alimentary Tract

Muscle strips of guinea pig stomach proved unresponsive even to 13-Nle-M concentrations of 0.5 × 10^{-6} g per ml. 13-Nle-M was also ineffective on guinea pig duodenum, terminal ileum, descending colon, and *Taenia coli* preparations. Also, there were no motor effects of 13-Nle-M (0.5 × 10^{-6} g per ml) on rat smooth muscle, including stomach, duodenum, terminal ileum, descending colon (circular and longitudinal muscle), and aortic strip; uterus preparations were found unresponsive even to 10 × 10^{-6} g of 13-Nle-M per ml.

Action of 13-Nle-M along the Human Alimentary Tract

Stomach. The threshold concentration of 13-Nle-M effective to cause increased contraction amplitude for body strips (n = 4) was found to be 50 × 10^{-9} g per ml. Antral strips (n = 5) already responded to 20 × 10^{-9} g of 13-Nle-M per ml. Figure 5 shows

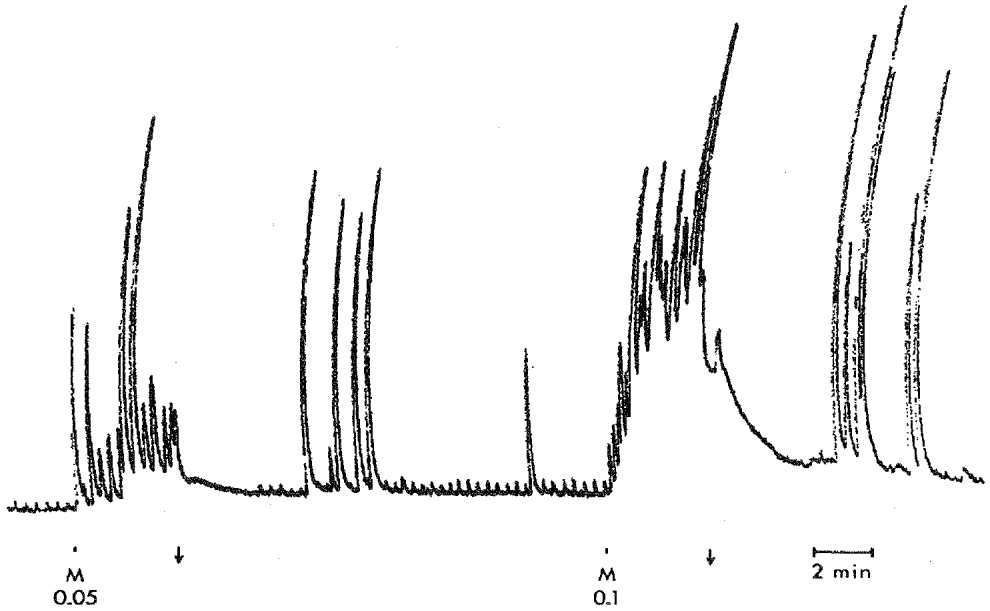

FIG. 3. Effects of 13-Nle-motilin (*M*), 0.05×10^{-6} and 0.1×10^{-6} g per ml, on antral circular muscle of rabbit stomach. Note spontaneous variations in basal rhythmicity.

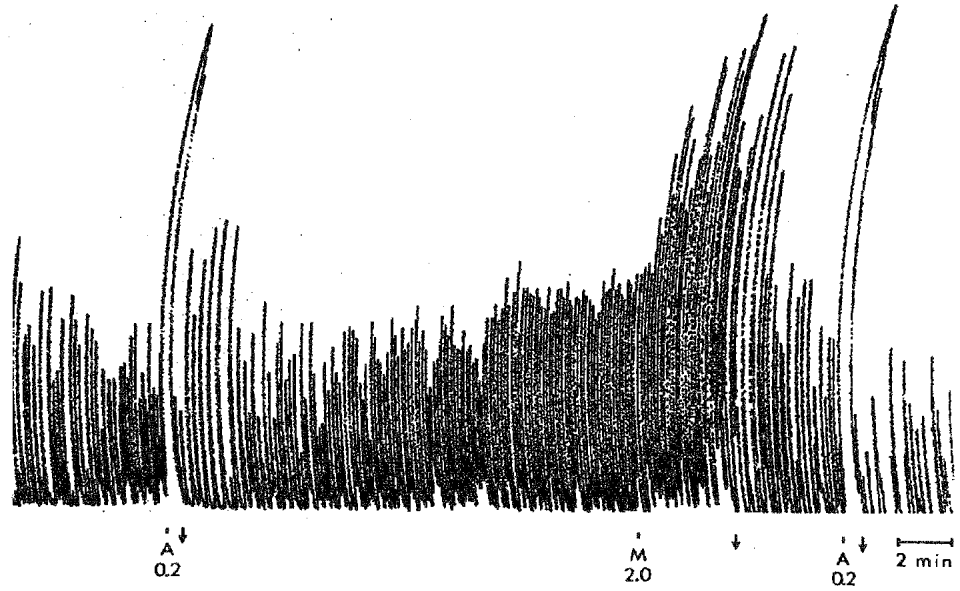

FIG. 4. Motility pattern of rabbit isolated terminal ileum due to acetylcholine (*A*, 0.2×10^{-6} g per ml) and to 13-Nle-motilin (*M*, 2.0×10^{-6} g per ml).

typical responses of an antral strip to increasing concentrations of 13-Nle-M.

Small intestine. Concentrations of 13-Nle-M already effective to contract circular muscle strips of the jejunum ($n = 9$) were 0.5×10^{-6} g per ml.

Large intestine. Strips of circular muscle of descending colon ($n = 8$), sigmoid colon ($n = 6$), and rectum ($n = 2$)—although highly sensitive to acetylcholine—proved unresponsive even to 0.5×10^{-6} g of 13-Nle-M per ml. By contrast, *Taenia coli*

preparations (n = 7) appeared to show small contractions to 0.5 × 10^{-6} g of 13-Nle-M per ml (fig. 6).

Gallbladder. Gallbladder muscle (n = 4) did not respond to 13-Nle-M (0.2 × 10^{-6} g per ml).

Pharmacological Analysis of Action of 13-Nle-M

Rabbit. Hexamethonium (20 × 10^{-6} g per ml) was shown to block the excitatory effects of nicotine (10 × 10^{-6} g per ml), but not to affect the response to 13-Nle-M (n = 4). Blockade of axonal conduction by tetrodotoxin (30 × 10^{-9} g per ml) likewise failed to inhibit 13-Nle-M actions; by contrast, contractile responses due to nicotine were abolished (fig. 7; n = 4). Atropine (10 × 10^{-6} g per ml) prevented the response to acetylcholine, but had no effect on the response to 13-Nle-M (n = 8).

The antihistaminic, pheniramine (10 × 10^{-6} g per ml), prevented histamine-induced contractions; however, the response to 13-Nle-M was not altered (n = 6).

Following administration of verapamil (2 × 10^{-6} g per ml), contractions by 13-Nle-M were abolished. The original susceptibility of the muscle strip to 13-Nle-M was almost fully restored after 30 min of washout (fig. 8; n = 9).

Man. In accordance with the preceding analysis, the contractile response of human gastric muscle to 13-Nle-M occurred in the presence of atropine (10^{-6} g per ml), whereas, as expected, the response to acetylcholine was abolished.

Discussion

In this study, 13-Nle-M has been shown to stimulate motor activity of muscle strips from both the rabbit and the human gastrointestinal tract, whereas preparations from the guinea pig and rat proved unresponsive. As to motor effects on rabbit isolated duodenum, 13-Nle-M exceeded the stimulatory activity of caerulein[9] by about 100 times (on molar basis).

Because human *Taenia coli* preparations seemed to show small contractions to 13-Nle-M, and because antral gastrin and gastric acid have been excluded as mediat-

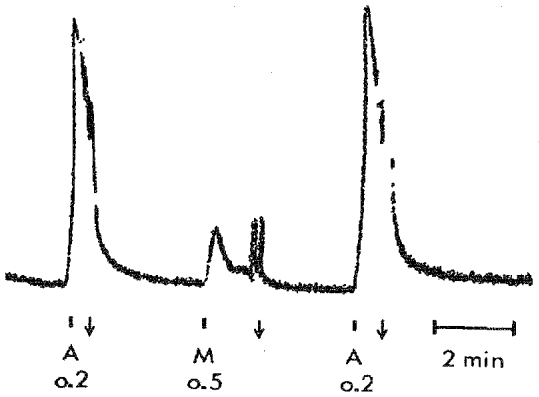

FIG. 6. Effects of 13-Nle-motilin (M, 0.5 × 10^{-6} g per ml) and acetylcholine (A, 0.2 × 10^{-6} g per ml) on isolated taenia of human sigmoid colon; representative recording.

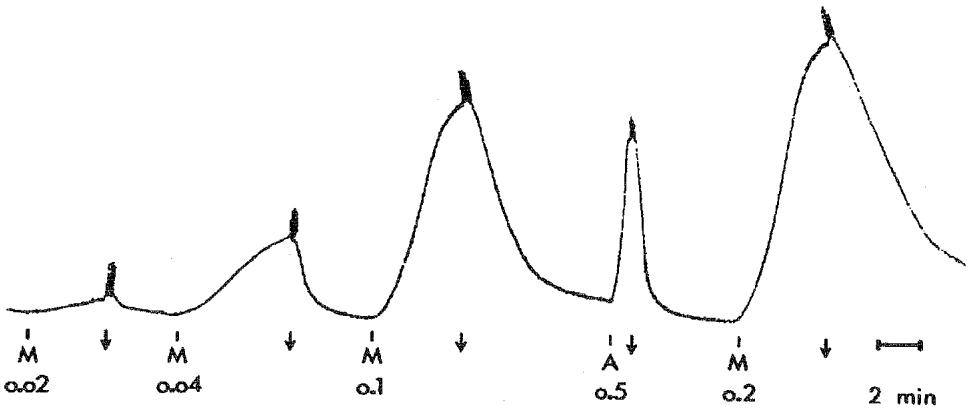

FIG. 5. Motility tracing of isolated antral circular muscle of human stomach. Responses to 13-Nle-motilin (M) and to acetylcholine (A). Concentrations are in 10^{-6} g per ml.

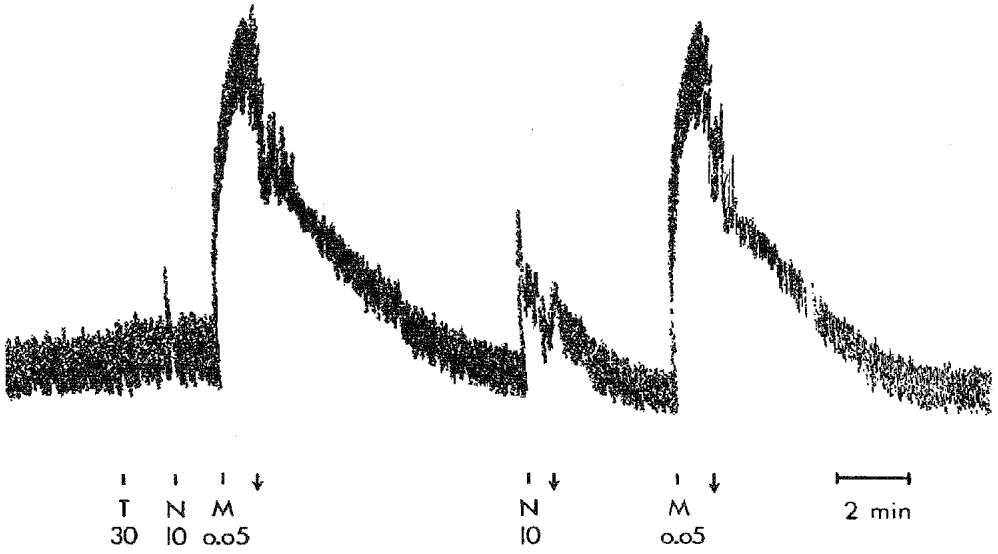

FIG. 7. Lack of effect of tetrodotoxin (T, 30 × 10^{-9} g per ml) on the response of isolated rabbit duodenum to 13-Nle-motilin (M, 0.05 × 10^{-6} g per ml). By contrast, tetrodotoxin inhibits contraction due to nicotine (N, 10 × 10^{-6} g per ml).

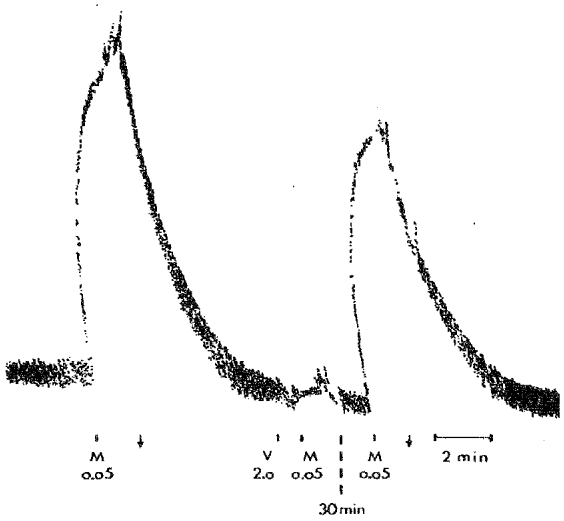

FIG. 8. Verapamil (V, 2.0 × 10^{-6} g per ml) abolishes the response of rabbit isolated duodenum to 13-Nle-motilin (M, 0.05 × 10^{-6} g per ml). Restoration of 13-Nle-motilin effect after 30 min of washout.

ing factors for the colonic motor response to a meal,[10] motilin may be speculated as a possible hormonal mediator of this reflex. However, in vivo observations are needed to clarify whether the colonic motor responses are the same when 13-Nle-M reaches the muscle through its vascular supply.

The pharmacological analysis of the mode of action of 13-Nle-M suggested that the polypeptide causes contractions by stimulating receptors on or in the muscle cell. An action on intrinsic nerves was excluded, since ganglion blockade by hexamethonium, blockade of axonal conduction by tetrodotoxin, or anticholinergic action by atropine did not alter the response of smooth muscle to 13-Nle-M. By use of the antihistaminic pheniramine, histamine receptors could be differentiated from the site of 13-Nle-M action. Verapamil, the only drug shown to abolish 13-Nle-M-induced contractions, was recently reported to exhibit Ca^{++} antagonistic properties preventing the action of Ca^{++} in excitation-contraction coupling.[8] Therefore it might be speculated that 13-Nle-M is involved in transport of Ca^{++} to the cytosol of intestinal smooth muscle, thus initiating contraction, but more evidence for this is needed.

The direct action of 13-Nle-M on gastrointestinal smooth muscle is different from the indirect action of gastrin- and cholecystokinin-like polypeptides which have been reported to contract longitudinal muscle strips of guinea pig ileum by releasing acetylcholine.[11, 12] In man, however,

gastrin has been shown to act directly on smooth muscle.[13] Thus, extrapolation of data from one to another species should not be done uncritically.[14] This is stressed by the fact that atropine was recently found to completely block the effects of motilin on mechanical activity of the isolated perfused canine stomach,[4] whereas in rabbit and man, the contractile response to 13-Nle-M is not at all altered by atropine.

The question now arises whether under physiological conditions motilin can act as a humoral factor influencing gastrointestinal motility. In favor of such a role for motilin the following can be considered: (1) the specificity of 13-Nle-M action, and (2) the high susceptibility of smooth muscle preparations from the rabbit and human upper gastrointestinal tract to the polypeptide. However, before hormonal status can be assigned to motilin, blood changes in motilin should be correlated with concurrent physiological changes.

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